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A one-tube method of reverse transcription-PCR to efficiently amplify a 3-kilobase region from the RNA polymerase gene to the poly(A) tail of small round-structured viruses (Norwalk-like viruses).

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Amplification of a 3-kb genome region from the RNA polymerase gene to the 3' poly(A) tail of small round-structured virus (SRSV) by reverse transcription-PCR (RT-PCR) has been difficult to achieve because of a stable secondary structure in a region between the RNA polymerase gene and the 5' end of the second open reading frame. We have developed a one-tube RT-PCR method to efficiently amplify this region. The method comprises three procedures: purification of poly(A)+ RNA from a starting RNA solution by oligo(dT)30 covalently linked to latex particles, buffer exchange, and continuous RT and PCR in a single tube containing all reaction components. The key elements of this method are (i) first-strand cDNA synthesis with the Superscript II version of RNase H- Moloney murine leukemia virus reverse transcriptase at 50 degrees C for 10 min by using the RNA-oligo(dT)30 hybrid on the latex particles as the template and primer, and (ii) PCR by Taq and Pwo DNA polymerases mixed together with a mixture of 12 phased oligo(dT)25 antisense primers. The detection threshold of the one-tube RT-PCR method was as little as 0.2 ng of the crude RNA used as the source of the template. Using this method, we obtained 3-kb products from 24 SRSV strains previously characterized into four genetic groups. These included 5 P1-A, 4 P1-B, 5 P2-A, and 10 P2-B strains. Because SRSVs have not yet been cultivated in vitro, this novel method should facilitate molecular characterization of SRSVs to provide a firm scientific foundation for improvements and refinements of SRSV diagnostics.

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